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(54) **EFFICIENT LIGHT HARVESTING**

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C12Q 1/02 (2006.01)
A01H 1/06 (2006.01)

(52) **U.S. Cl.**

CPC .. ***C12Q 1/02*** (2013.01); ***A01H 1/06*** (2013.01); *G01N 2333/405* (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

Various aspects provide for genetically modifying photosynthetic cells. In some cases, an integrated light harvesting efficiency of photosynthetic cells may be increased by reducing the amount of incident light that is absorbed but not used for photosynthesis. In some cases, an increased transparency may be associated with an increased light harvesting efficiency when absorption due to non-photosynthetic processes is reduced. A reduced capacity of various light-harvesting antenna apparatus may increase transparency. In some cases, a capacity of an organism to adapt to varying light levels may be reduced, and in certain cases, a modified organism may have a reduced ability to acclimate to a low light irradiance.

13 Claims, 18 Drawing Sheets

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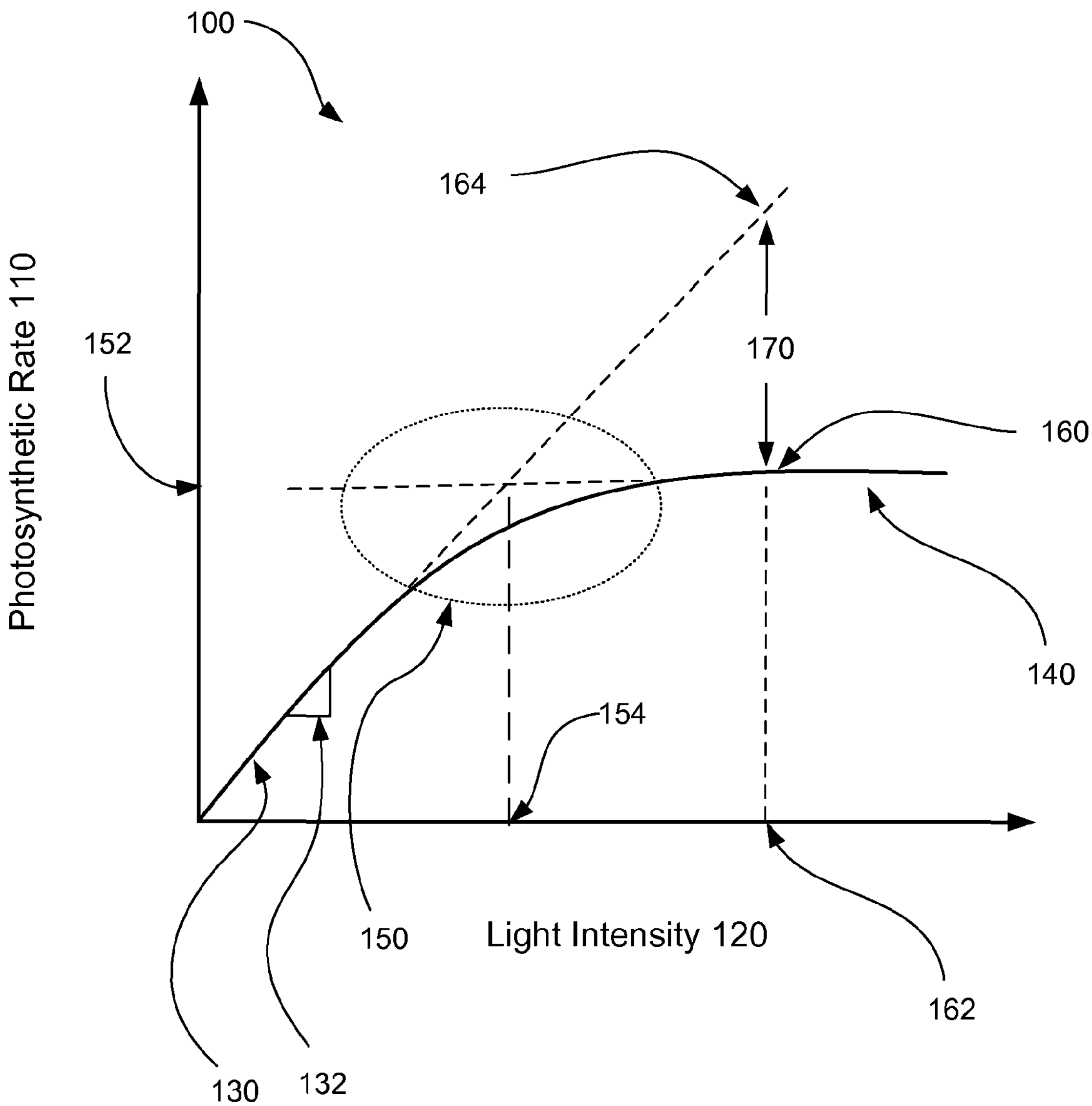
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Light Intensity 120
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FIG. 1

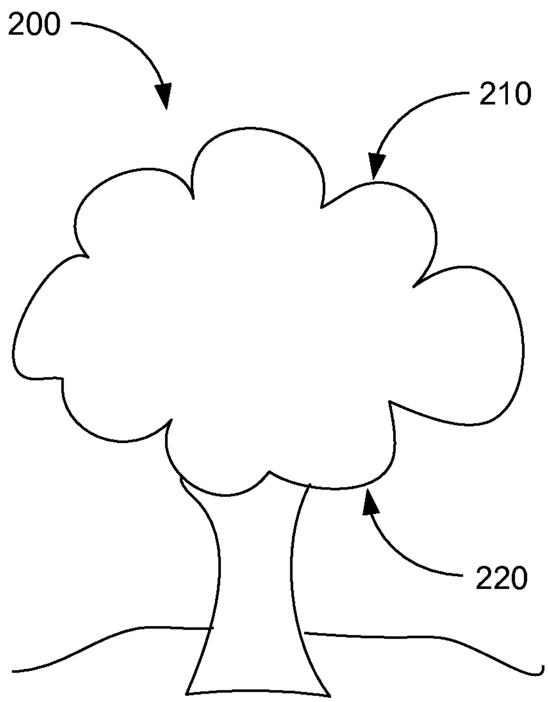

FIG. 2A

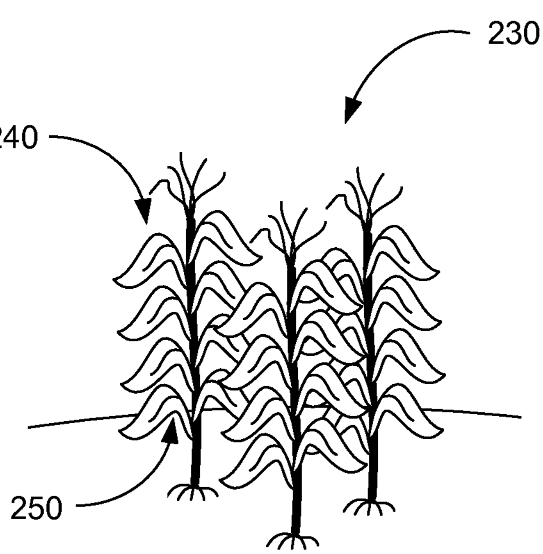

FIG. 2B

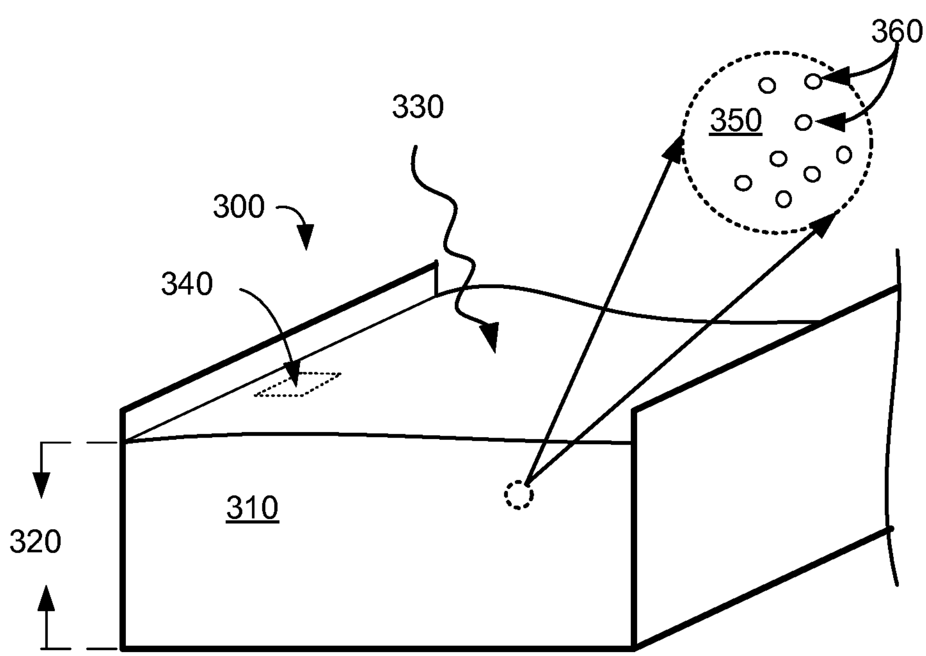
360
330
350
300
340
310
320

FIG. 3A

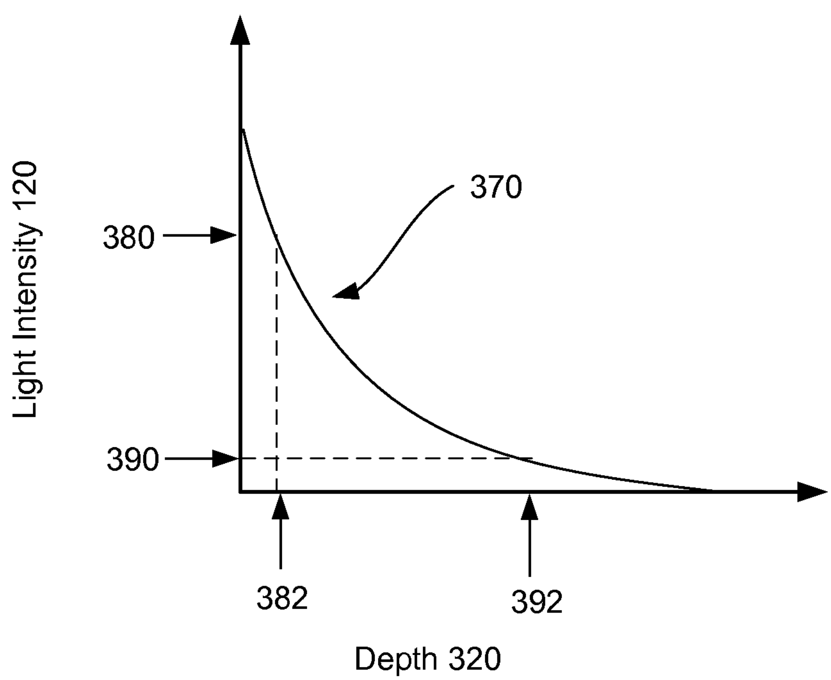
Light Intensity 120
370
380
390
382
392
Depth 320

FIG. 3B

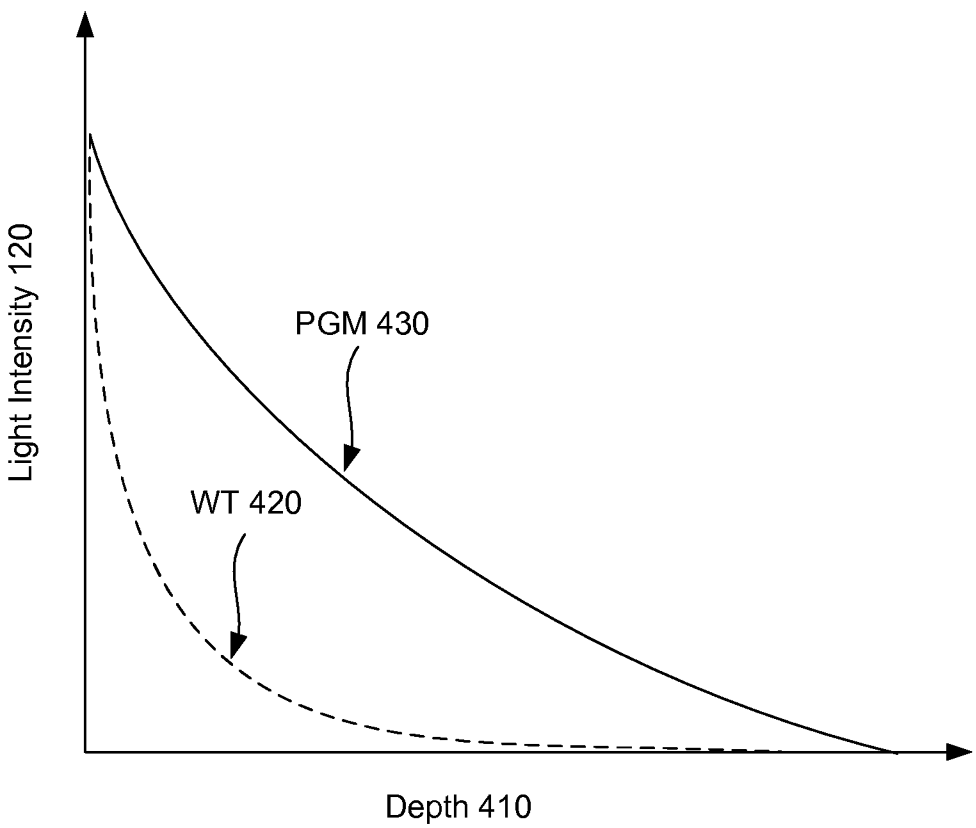

FIG. 4

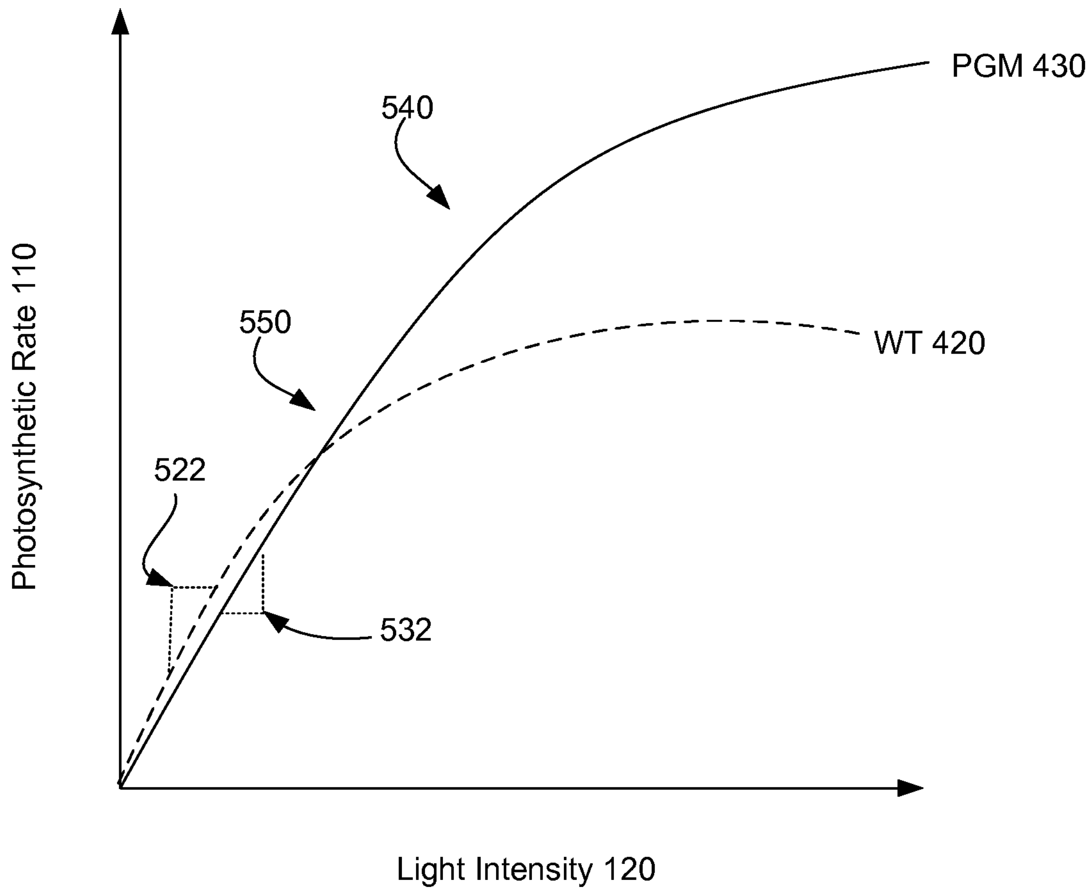
PGM 430
540
Photosynthetic Rate 110
550
WT 420
522
532
Light Intensity 120

FIG. 5

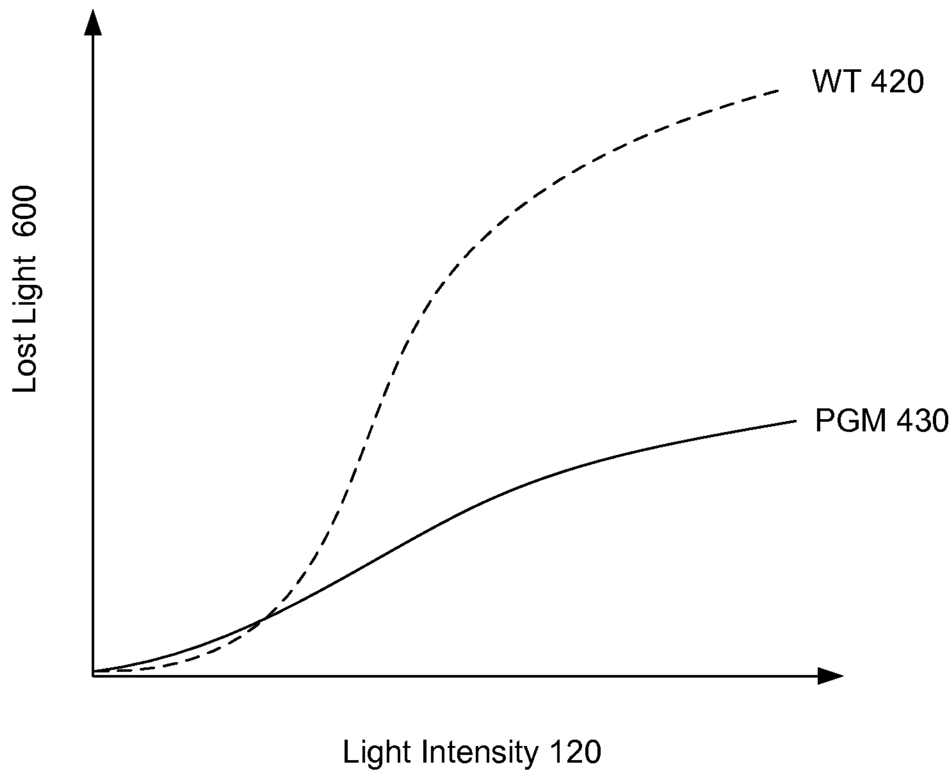

FIG. 6

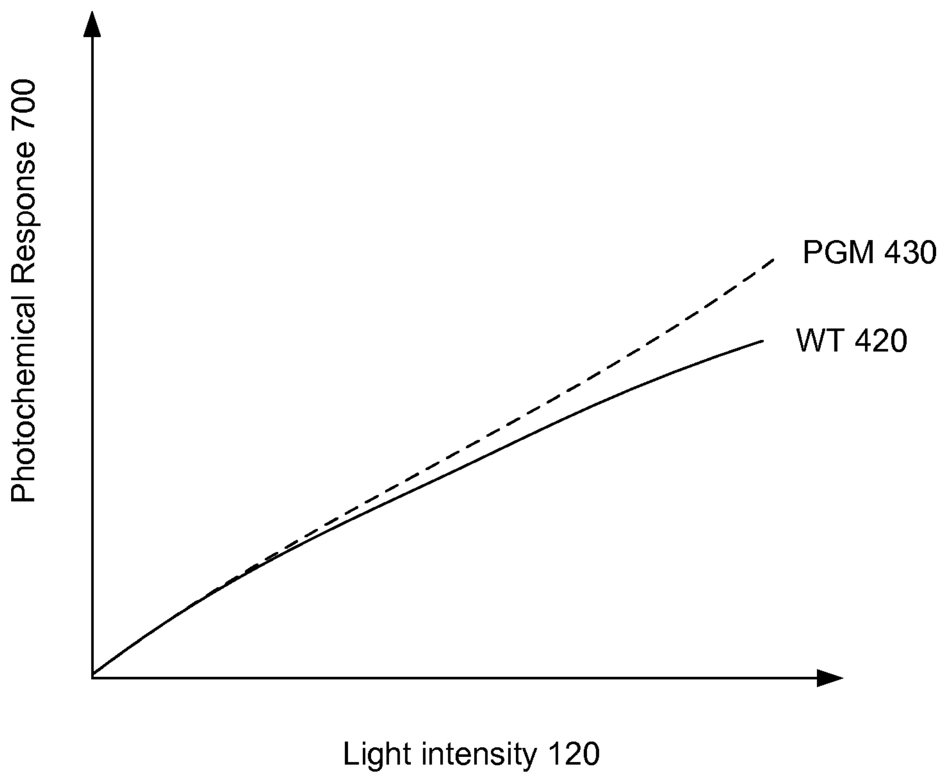
Photochemical Response 700
PGM 430
WT 420
Light intensity 120

FIG. 7

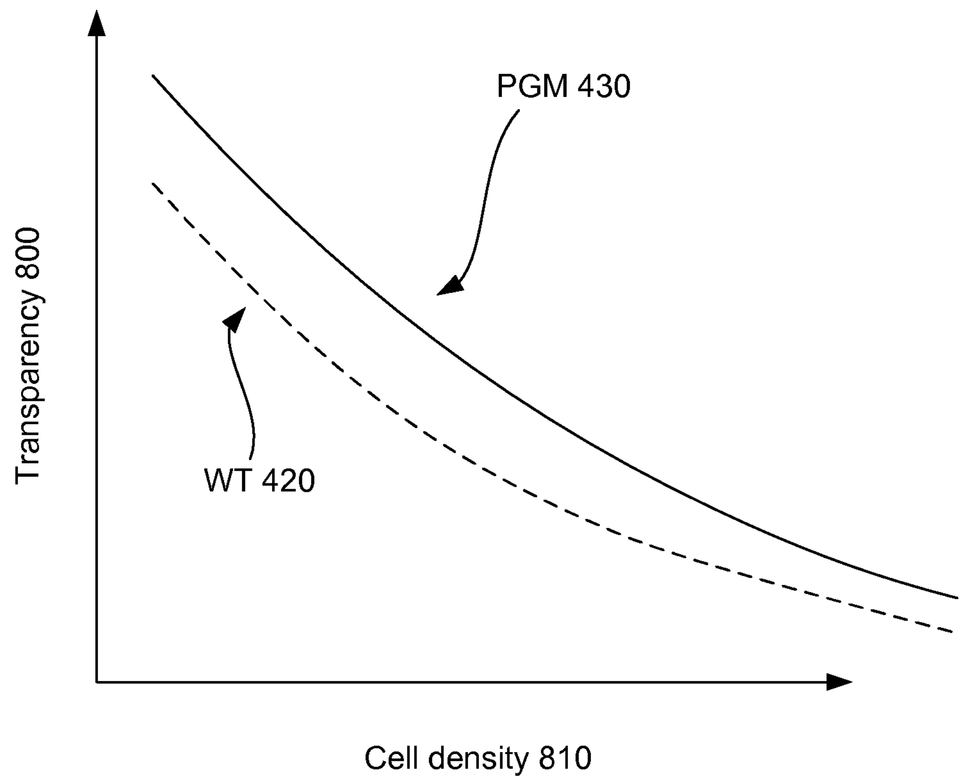

FIG. 8

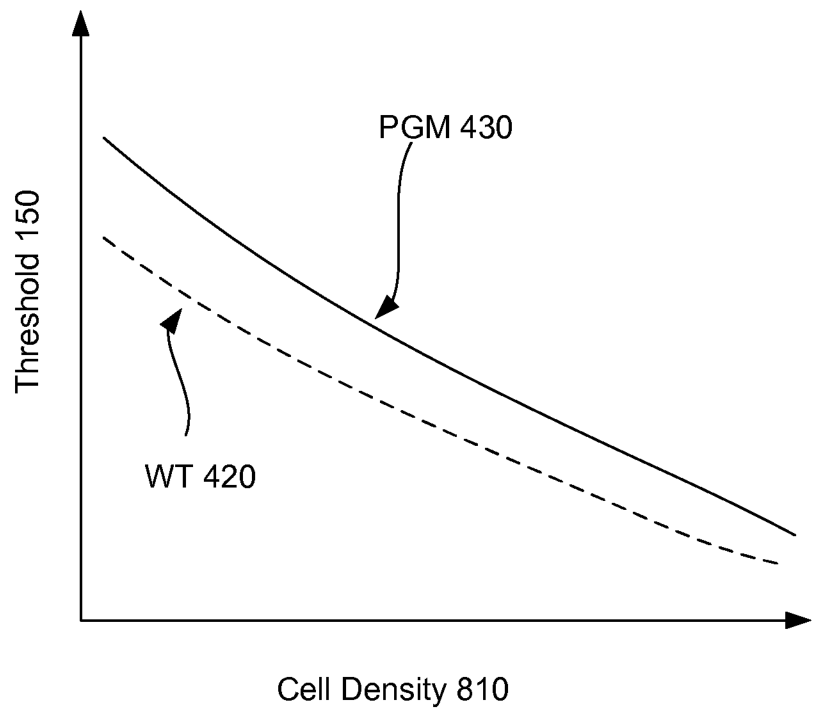

FIG.9

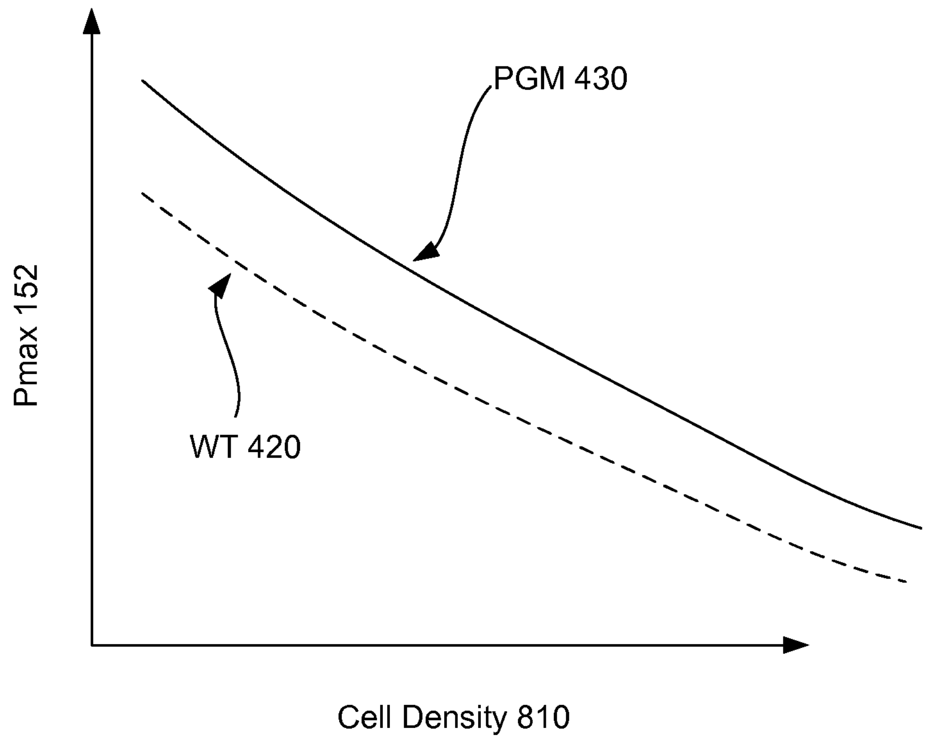

FIG. 10

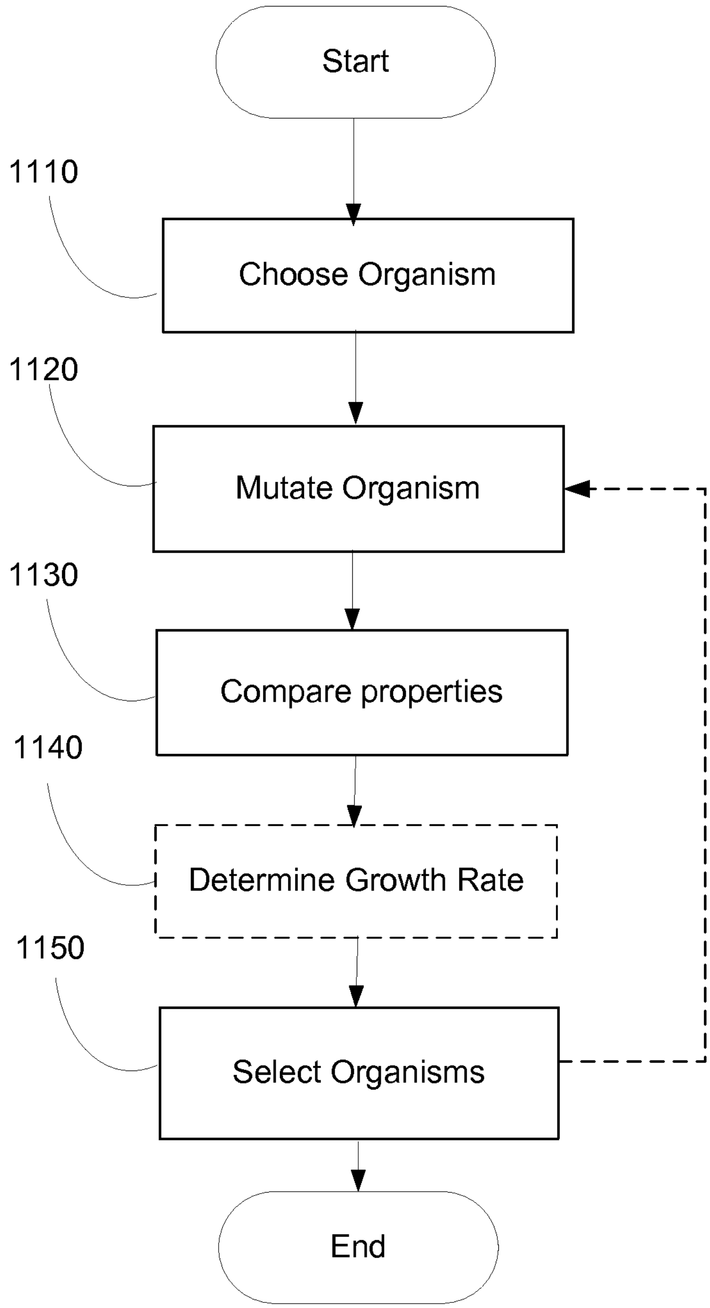
Start
1110
Choose Organism
1120
Mutate Organism
1130
Compare properties
1140
Determine Growth Rate
1150
Select Organisms
End

FIG. 11

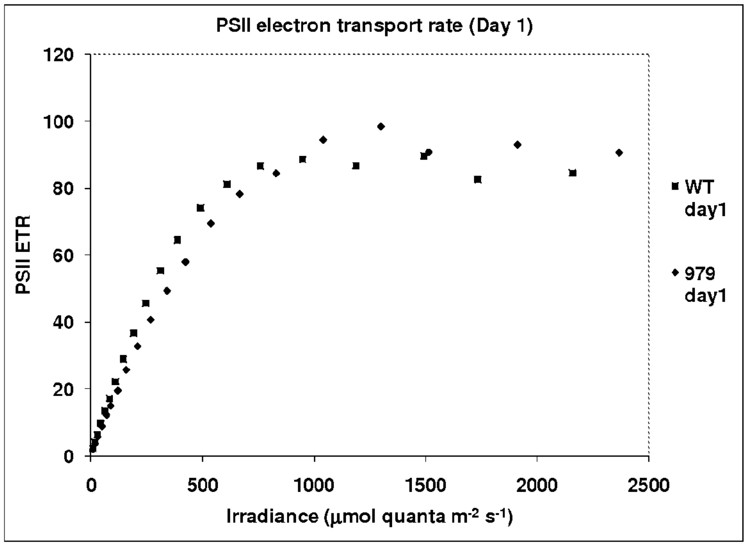

FIG. 12A

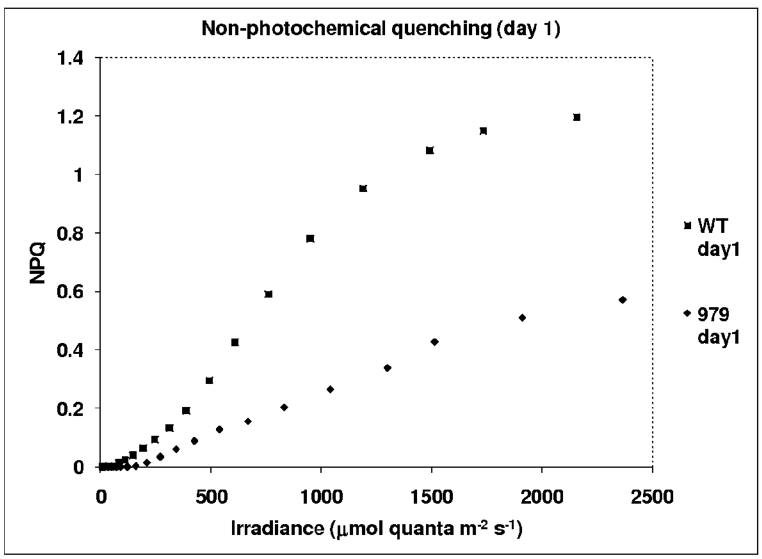

FIG. 12B

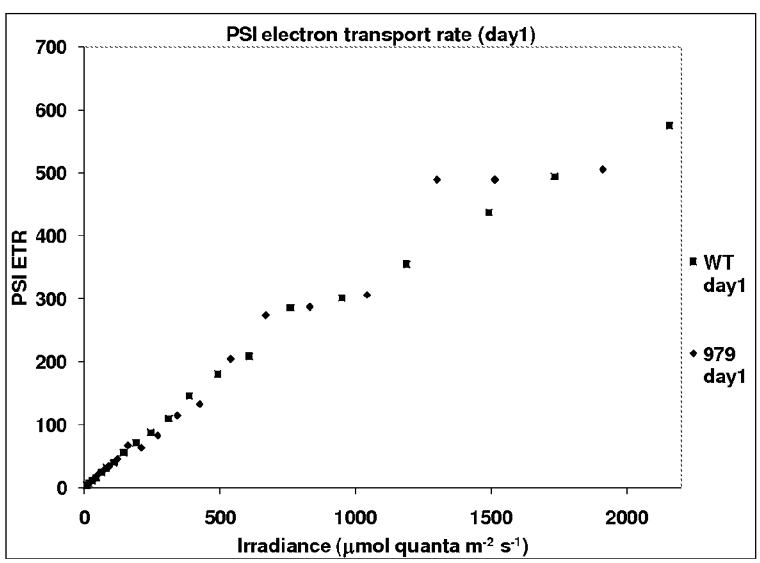

FIG. 12C

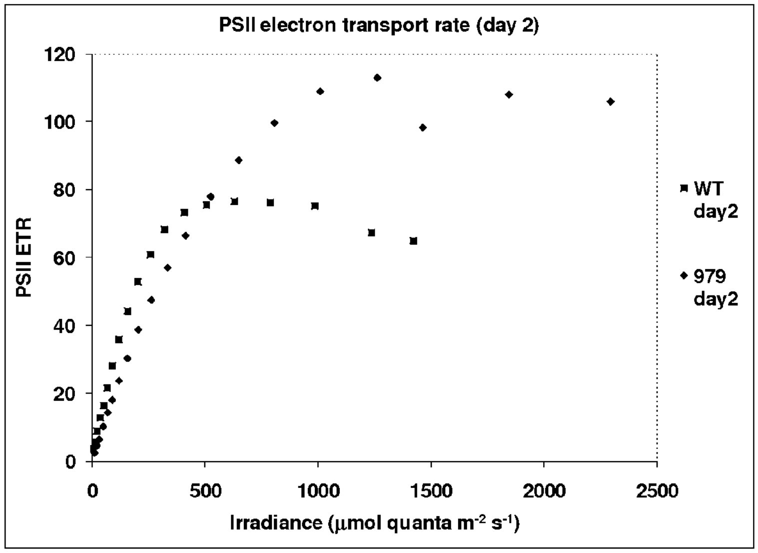

FIG. 13A

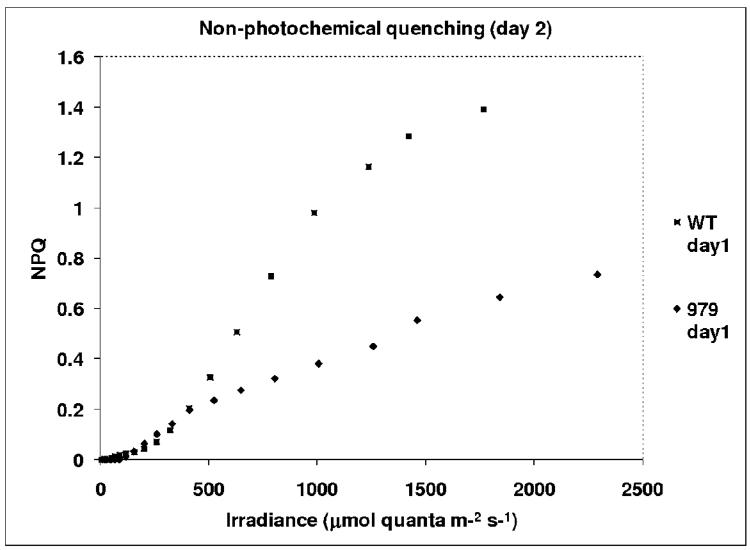

FIG. 13B

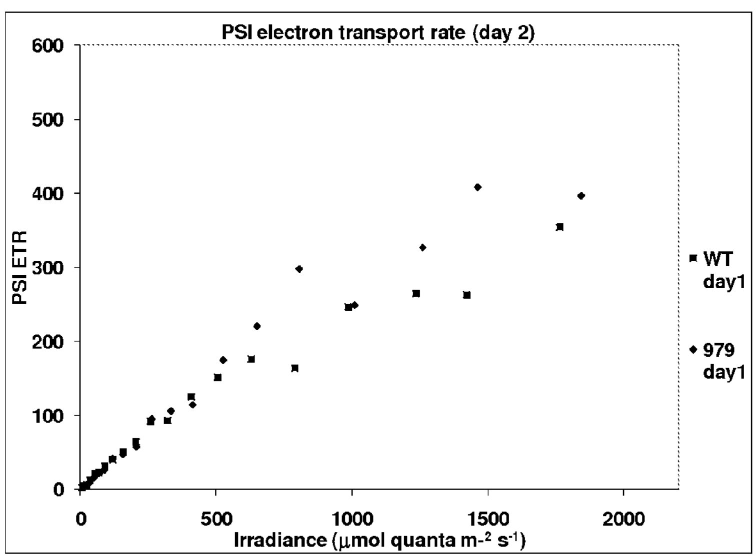

FIG. 13C

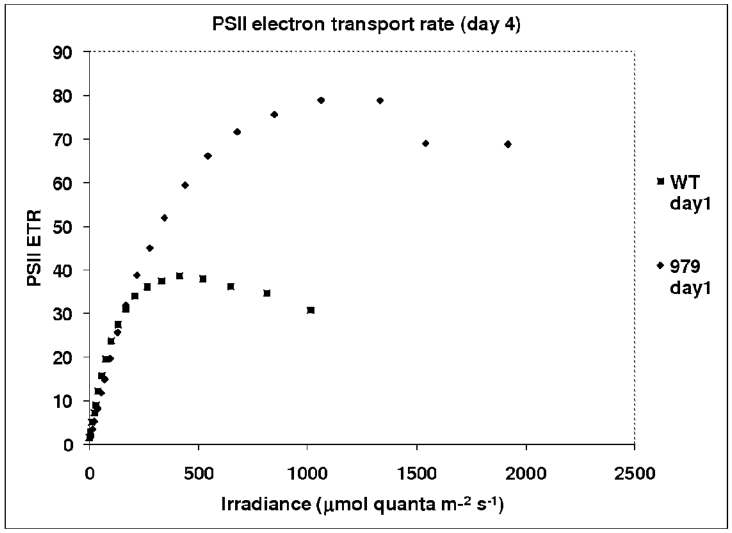

FIG. 14A

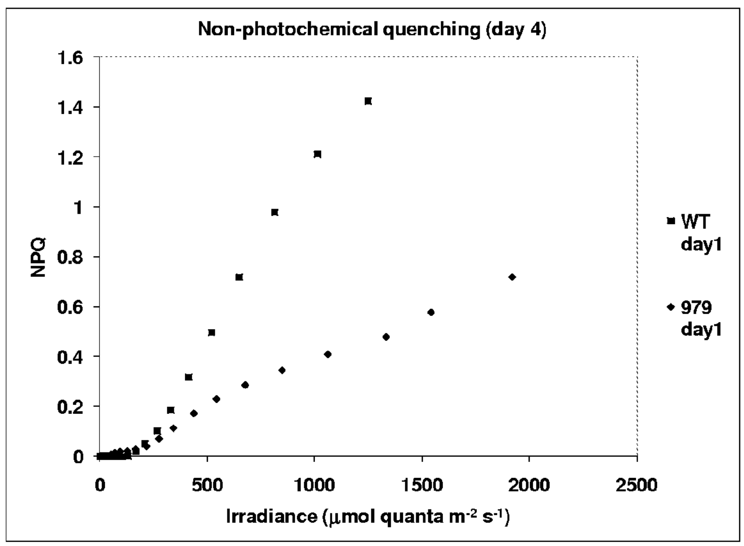

FIG. 14B

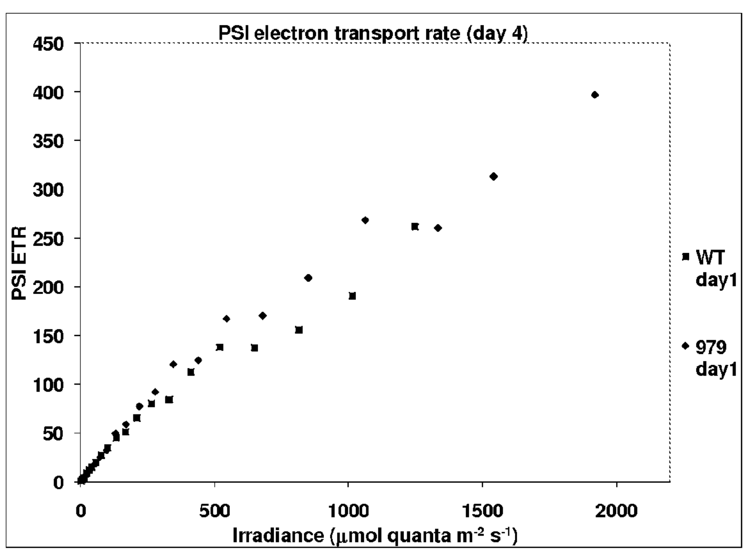

FIG. 14C

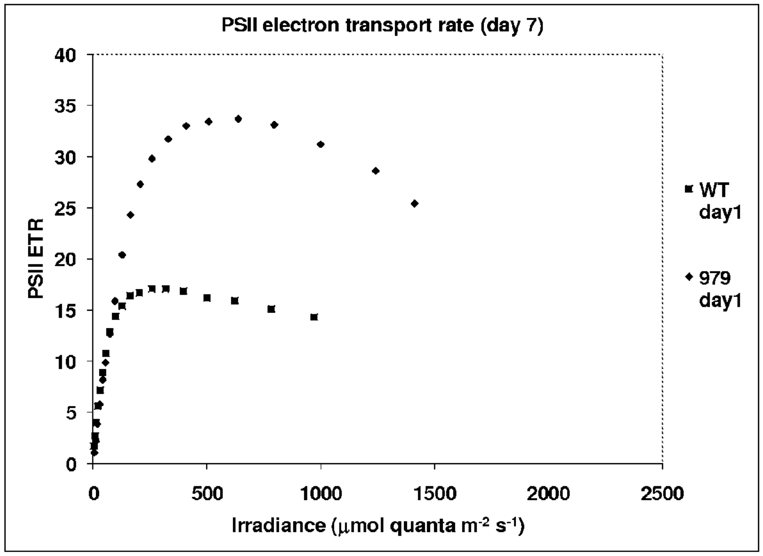

FIG. 15A

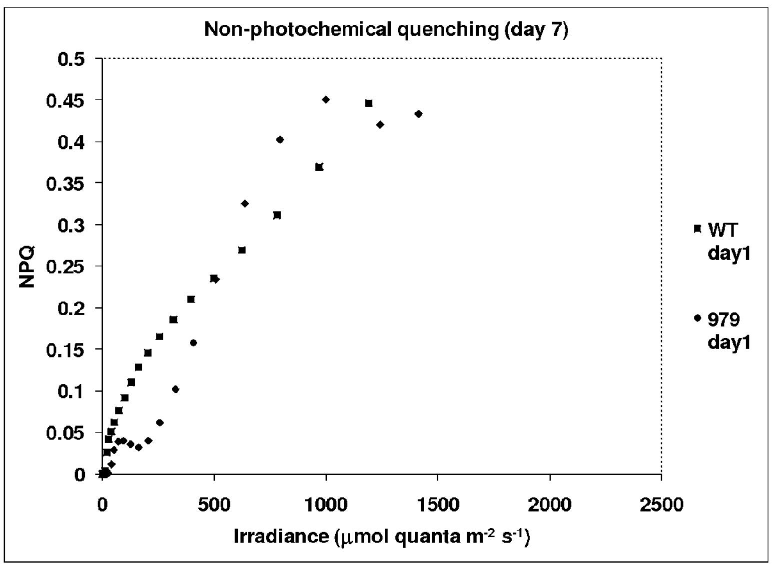

FIG. 15B

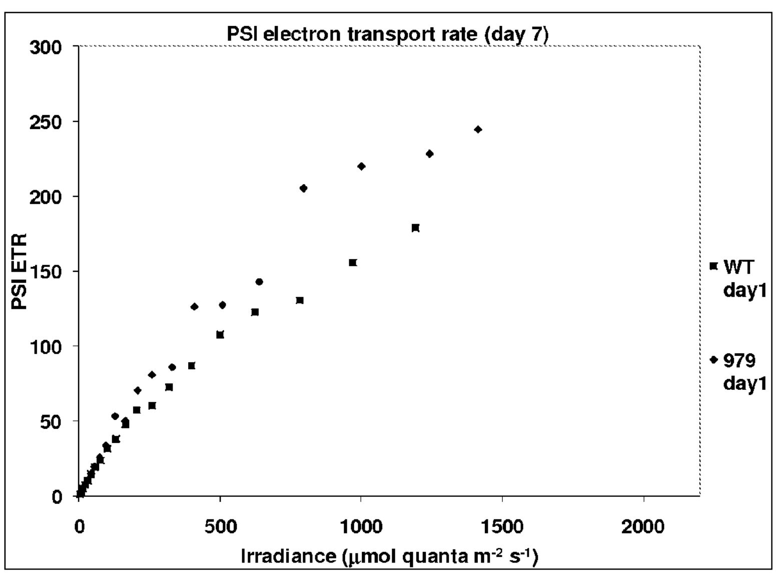

FIG. 15C

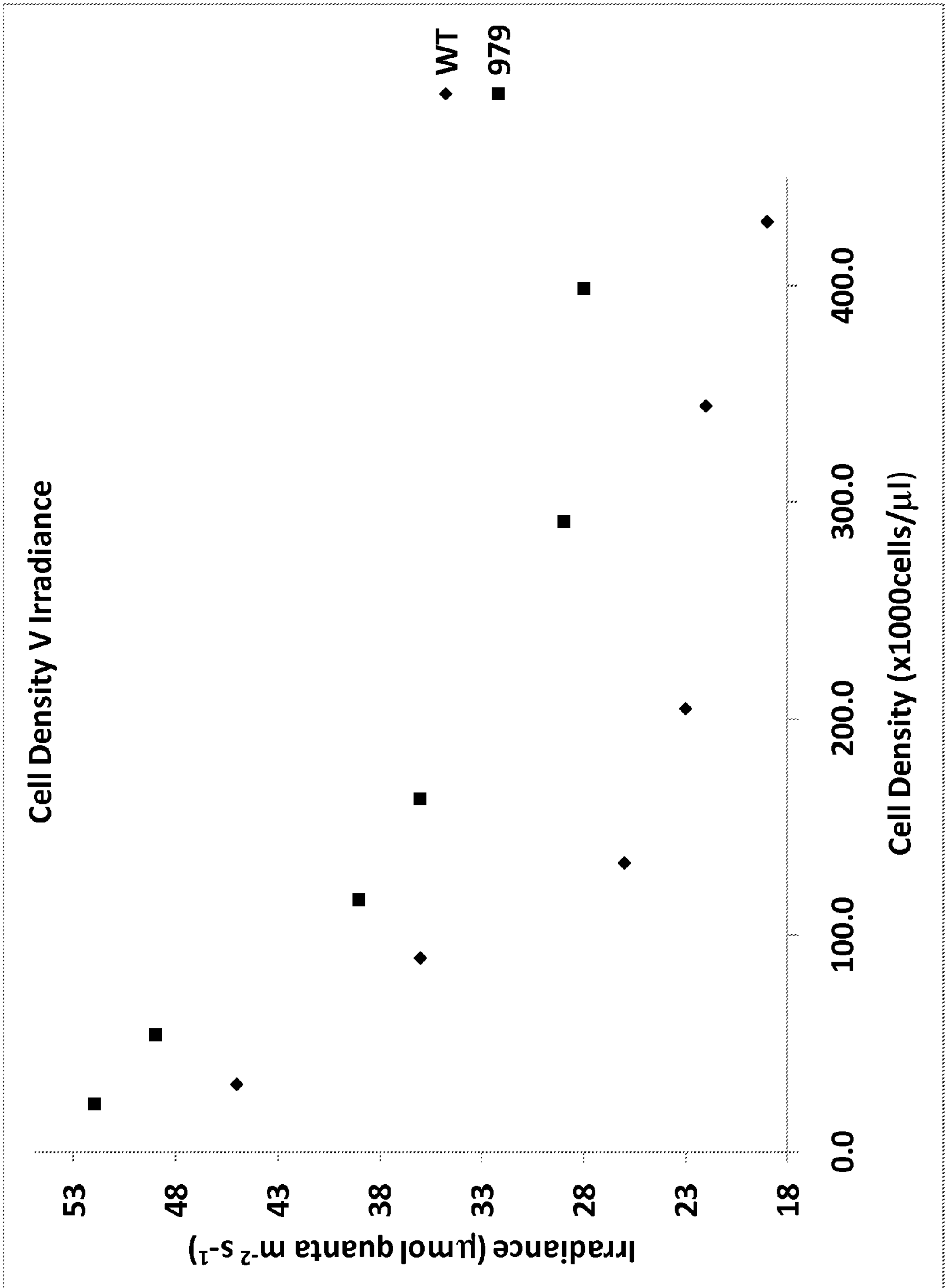
Cell Density V Irradiance
WT
979
Irradiance (µmol quanta m-2 s-1)
53
48
43
38
33
28
23
18
0.0
100.0
200.0
300.0
400.0
Cell Density (x1000cells/µl)

FIG. 16

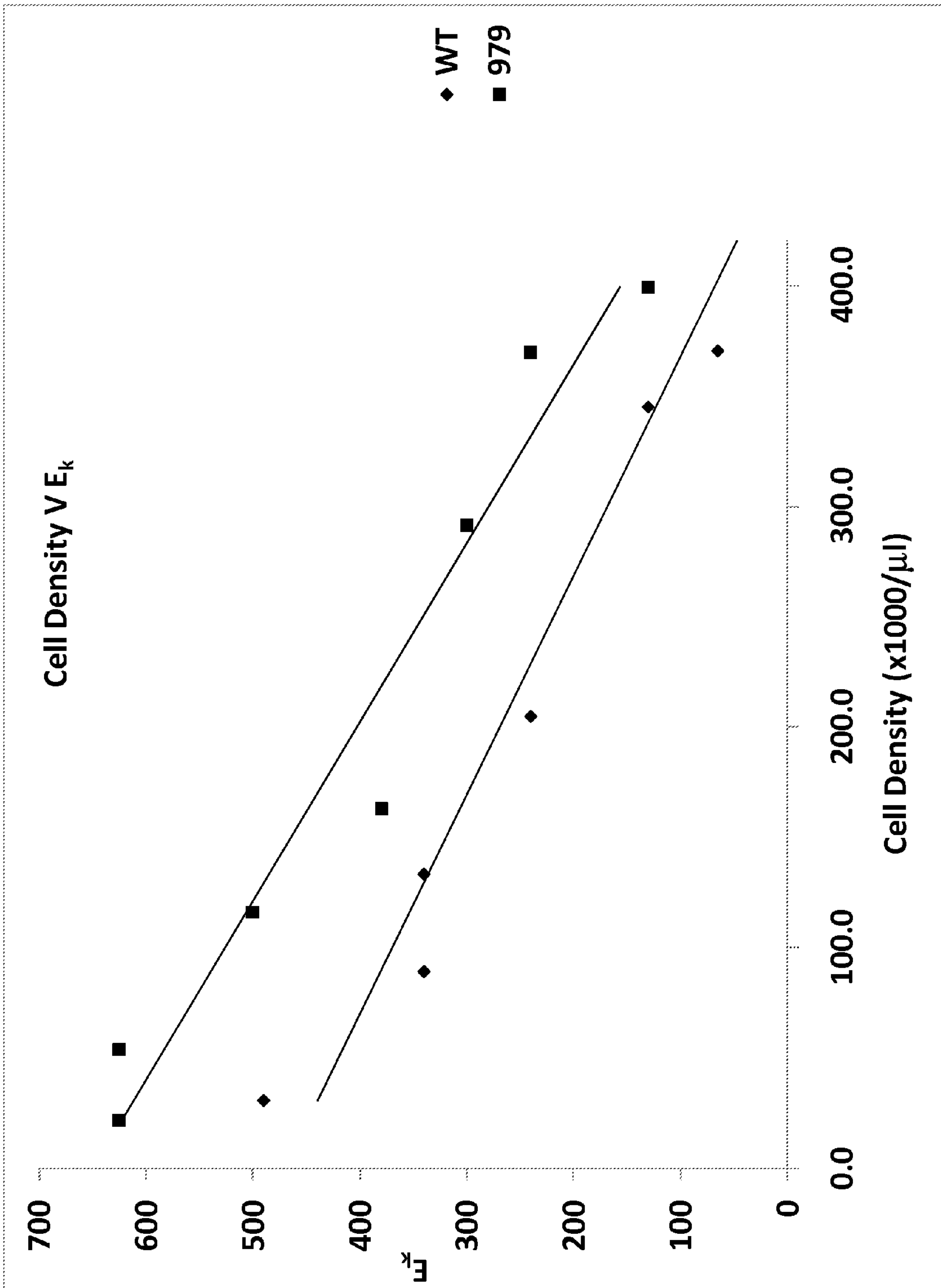
Cell Density V E_k
WT
979
E_k
700
600
500
400
300
200
100
0
0.0
100.0
200.0
300.0
400.0
Cell Density (x1000/μl)

FIG. 17

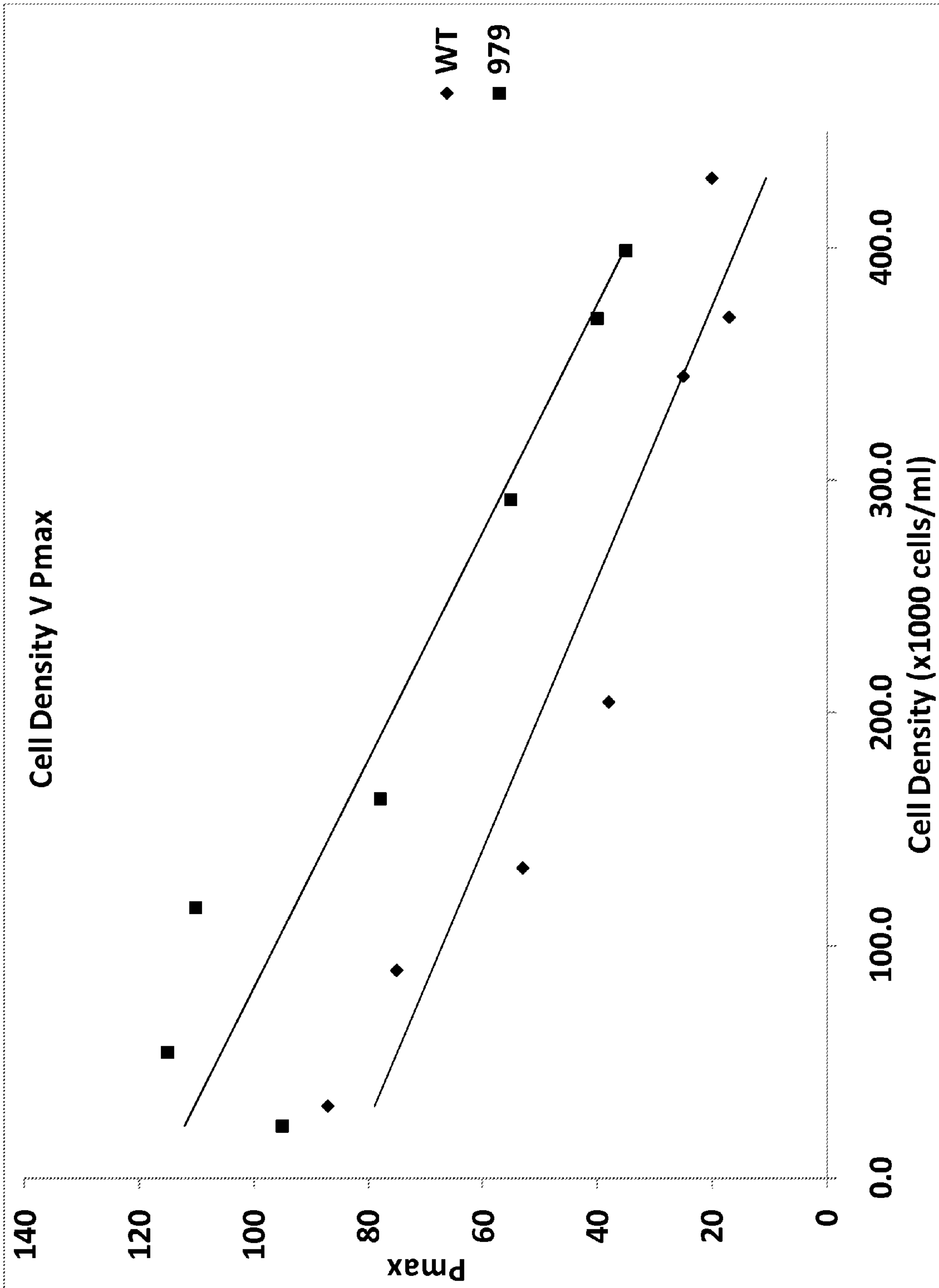
Cell Density V Pmax
WT
979
Pmax
140
120
100
80
60
40
20
0
0.0
100.0
200.0
300.0
400.0
Cell Density (x1000 cells/ml)

FIG. 18

EFFICIENT LIGHT HARVESTING

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the priority benefit of U.S. provisional patent application No. 61/175,444, filed May 4, 2009.

BACKGROUND

1. Technical Field

The present invention relates to improving the efficiency with which photosynthetic organisms use light.

2. Description of Related Art

Photosynthetic organisms use energy from light to form chemical bonds. Energy embodied within the chemical bonds may be used at a later date. As such, chemical bonds provide a storage mechanism for the energy associated with incident light.

A supply of light is often finite in a given period of time. For a given fluence of incident light, photosynthetic organisms may use some of the light to perform photosynthesis. Some of the light may not be used for photosynthesis. Some light may be converted to heat. Some light may be absorbed and reemitted (e.g., fluoresced). Some light may damage the organism. Light that is not used for photosynthesis may not be converted into stored chemical energy within the organism, and so the energy associated with this unconverted light may not be available for subsequent use. Improving the conversion of incident light to biomass (e.g., increasing the percentage of incident energy converted to chemical bonds) may increase the efficiency of biomass production, which may increase the amount of incident solar energy available for subsequent use.

SUMMARY OF THE INVENTION

Various aspects provide for selecting a natural and/or wild type photosynthetic organism. Cells of the wild type organism may have a first transparency associated with light transmission through the cells. The organism may be subject to mutagenesis to create one or more mutated photosynthetic organisms. Transparencies of the cells of the mutated organisms may be determined, and a mutated organism having a transparency greater than that of the wild type organism may be selected.

In some cases, growth rates may be measured. A mutated organism and/or a plurality of mutated organisms (e.g., a suspension of organisms) may have a higher growth rate than a similar wild type. In some cases, more transparent organisms or cells may have a higher overall growth rate. Growth rate may be measured in terms of total biomass (e.g., dry matter) and/or quantities of certain components or chemicals (carbohydrates, proteins, lipids, nucleic acids, and the like). Growth rate may include or be normalized to a quantity of incident radiation (e.g., light or sunlight).

Organisms may include crops such as corn, rice, wheat, sugarcane, and the like. Organisms may include trees, such as poplar, conifers, jatropha, palm, and the like. Organisms may include grasses such as prairie grasses, switchgrass, *Miscanthus*, and the like. Organisms may include single cell organisms such as algae, diatoms, cyanobacteria, and the like.

In some cases, mutated organisms may be identified optically, for example using fluorescence. In certain cases, a more transparent organism may have a paler green color than a less transparent version. Organisms may be identified using various responses, such as a photosystem I response, photosys-

tem II response, nonphotochemical quenching, photosynthetic rate, irradiance threshold, and the like.

In some embodiments, an organism may be modified to reduce a sensitivity of one or more light harvesting apparatus and/or mechanisms. In some cases, a light harvesting antenna (e.g., associated with photosystem II) may be modified to have a reduced effectiveness or efficiency as compared to an unmodified (e.g., wild type) organism. In some cases, modification of an organism may result in a modified organism having a reduced ability to adapt to changing light conditions. In certain examples, this reduced ability may be manifest as a reduced ability to adapt to low light conditions. Certain cells and/or organisms may be described as being "locked" into an acclimation state associated with high irradiance levels, despite exposure to low irradiance levels.

Organisms may be mutated (e.g., using mutagenesis) to create one or more mutated versions of the organism. Mutated versions may be screened for one or more properties. In some cases, a plurality of mutated organisms (e.g., a suspension of algae or diatoms) may have an increased transparency and a higher growth rate than an otherwise equivalent plurality of wild type organisms.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** illustrates an exemplary saturation response of a photosynthetic cell, according to some embodiments.

FIGS. **2**A and **2**B illustrate variations in light intensity for exemplary pluralities of photosynthetic cells.

FIG. **3**A illustrates an exemplary suspension.

FIG. **3**B is a schematic illustration of an exemplary variation in light intensity with depth through a plurality of cells (e.g., within a suspension).

FIG. **4** is a schematic illustration of an effect of increased transparency, according to some embodiments.

FIG. **5** illustrates a schematic comparison of two photosynthetic rate responses, according to some embodiments.

FIG. **6** illustrates a schematic comparison of light loss as a function of light intensity, according to some embodiments.

FIG. **7** illustrates a schematic comparison of photochemical responses, according to some embodiments.

FIG. **8** illustrates a schematic comparison of transparency as a function of cell density, according to some embodiments.

FIG. **9** illustrates a schematic comparison of the transition thresholds of modified and unmodified cells, according to some embodiments.

FIG. **10** illustrates a schematic comparison of maximum photosynthetic rates, according to certain embodiments.

FIG. **11** illustrates an exemplary method.

FIGS. **12**A, **12**B, and **12**C illustrate experimental results for wild type (annotated WT) and mutated (annotated **979**) samples after day 1 of growth.

FIGS. **13**A, **13**B, and **13**C illustrate experimental results for wild type (annotated WT) and mutated (annotated **979**) samples after day 2 of growth.

FIGS. **14**A, **14**B, and **14**C illustrate experimental results for wild type (annotated WT) and mutated (annotated **979**) samples after day 4 of growth.

FIGS. **15**A, **15**B, and **15**C illustrate experimental results for wild type (annotated WT) and mutated (annotated **979**) samples after day 7 of growth.

FIG. **16** illustrates a comparison of irradiance vs. cell density for wild type (annotated WT) and mutated (annotated **979**) samples.

FIG. **17** illustrates a comparison of measured Ek vs. cell density for wild type (annotated WT) and mutated (annotated **979**) samples.

FIG. **18** illustrates a comparison of measured Pmax vs. cell density for wild type (annotated WT) and mutated (annotated **979**) samples.

DETAILED DESCRIPTION OF THE INVENTION

Many organisms include cells, organelles, membranes, and the like that perform photosynthesis. A photosynthetic cell may be modified to change (e.g., increase or decrease) its transparency to light. Modification of a cell may include mutating the cell, and may include performing PCR Mutagenesis, Transposon Mutagenesis, Site-directed Mutagenesis, Directed Mutagenesis, Random Mutagenesis, Insertional Mutagenesis, Targeted Mutagenesis, and the like on the cell.

Transparency may be changed by modifying the size of a light harvesting antenna (LHA). In some cases, LHA associated with Photosystem II (PSII) may be modified in a manner that increases transparency. A reduced transparency of a first cell may result in a greater amount of light passing through the first cell to a second cell. The second cell may productively utilize a portion of light that might have been dissipated by the first cell were it not to pass through the first cell.

A plurality of cells having increased transparency may have a higher overall photochemical efficiency than a similar plurality having reduced (e.g., native) transparency. A light harvesting efficiency of a population of photosynthetic cells may be increased by reducing the total amount of incident light absorbed, scattered, converted, or otherwise consumed by non-photosynthetic reactions. In some cases, an overall or integrated growth rate of the group of more transparent cells may be as great as, or even greater than, the growth rate of the group of less transparent cells. In some cases, a more transparent cell may be less susceptible to damage, particularly under bright light conditions. A more transparent cell may be more robust to changing light conditions (e.g., passing from a low light condition to a high light condition).

An efficiency with which light is harvested by a group of photosynthetic cells may increase the amount of incident energy that is converted to chemical bonds. An increase in light harvesting efficiency may be manifest in a reduction in the incident energy needed to create biomass, which may be manifest as a concomitant reduction in the energy needed to produce biomass-derived products, such as biochemicals, biofuels, ethanol, esters, alkanes, nutrients, food, supplements, and/or other products derived from photosynthetic organisms.

Many photosynthetic cells have a finite capacity to utilize incident light for photosynthesis. A low intensity light may be efficiently utilized (e.g., substantially converted to chemical energy, or converted as efficiently as quantum or physiological limits allow). A more intense light may "overpower" the organism's photosynthesis capabilities, resulting in a substantial portion of the incident light not being used for photosynthesis. Such unused light may be absorbed, create heat, damage the organism, or may otherwise be "wasted." In some cases, high intensity light may damage a cell in a way that results in decreased photosynthetic efficiency, decreased growth rate, or even death of the cell.

FIG. **1** illustrates an exemplary saturation response of a photosynthetic cell, according to some embodiments. FIG. **1** illustrates a schematic response **100** describing photosynthetic rate **110** as a function of light intensity **120**. Photosynthetic rate **110** may represent or be represented by photosynthetic productivity, photosynthetic efficiency, electron transport rates, lipid productivity, biomass productivity, oxygen production, CO2 sequestration, and the like. Photosynthetic rate **110** may be associated with Photosystem I and/or II production. In some cases, photosynthetic rate **110** may represent an electron transport rate associated with PSII.

Response **100** may include a substantially "linear" regime **130** and a saturation regime **140**. Linear regime **130** and saturation regime **140** may be separated by a threshold **150**. Threshold **150** may be broad or narrow, and may be empirically associated with a transition between regimes. Threshold **150** may vary among diverse photosynthetic organisms—trees, grasses, corn, sugarcane, algae, diatoms, rhizomes such as switchgrass (*Panicum*), prairie grass (e.g., *Miscanthus*), and the like. For some algae (e.g., *Nannochloropsis*), a threshold **150** may be near 200 μmol quanta/m^2-sec. In some cases, a relatively "maximum" photosynthetic rate Pmax **152** may be defined. Threshold **150** may be associated with a light intensity such as Ek **154**, which may represent an irradiance level at which an optimum photosynthetic rate is achieved.

Linear regime **130** may be associated with a region of light intensity in which photosynthetic rate **110** is approximately linearly dependent upon light intensity **120**. A linear regime **130** may be characterized as a "light limited" regime, in that photosynthetic productivity is ostensibly limited by the available light, not by the cell per se. In some cases, an organism may be characterized by a slope **132** associated with linear regime **130**. For some organisms, slope **132** of the photosynthetic rate vs. intensity response may be associated with a quantum yield of Photosystem II photochemistry. Slope **132** may be characterized by one or more metrics, (e.g., moles of O2 evolved per number of incident photons, mass of CO2 converted to biomass per input energy, and the like).

Saturation regime **140** may be characterized by a photosynthetic rate below what would be expected based on an extrapolation of the response in linear regime **130** (to higher intensities). For example, an observed photosynthetic rate **160** at intensity **162** may be below an extrapolated photosynthetic rate **164** (based on extrapolating from linear regime **130**, e.g. using slope **132**). An organism receiving an intensity in saturation regime **140** (e.g., at intensity **162**) may use a relatively smaller percentage of the incident light for photosynthesis, as compared to an organism in linear regime **130**. Such an exposure may overwhelm the photosynthetic capabilities of the organism, resulting in a relatively larger amount of the light not being utilized for photosynthesis. Such an exposure may be characterized by a lost productivity **170**, which may be associated with a difference between actual photosynthetic rate and a photosynthetic rate that might be expected based on a productivity response at lower intensities (e.g., in a light limited regime).

Photosynthetic cells may be exposed to a wide range of light intensities. In some cases, a single organism may have some cells that are exposed to bright light, while other cells are exposed to weaker light. In some cases, single cells or single celled organisms (e.g., algae, diatoms, and the like) may be exposed to a range of light intensities. For example, algae in water may circulate from the surface (where light is intense) to a depth beneath the surface at which light is faint. Certain embodiments include maximizing a number of organisms exposed to an intensity near (or below) threshold **150**.

FIGS. **2**A and **2**B illustrate variations in light intensity for exemplary pluralities of photosynthetic cells. FIG. **2**A illustrates a tree **200** having some cells **210** exposed to bright light, and other cells **220** exposed to weaker light. In some cases, cells **210** may at least partially shade cells **220**. Exemplary organisms include firs, pines, poplars, and other plants.

FIG. **2**B illustrates a plurality of photosynthetic organisms having cells exposed to a different light intensities. Crop **230** may include one or more plants for which some cells **240** are exposed to more intense light, and other cells **250** are exposed to less intense light. Exemplary crops **230** include corn, oats, wheat, barley, rice, sugarcane, beets, bamboo, palm, jatropha, various grasses such as prairie grasses, halophytes (e.g., *Spartina, Salicornia*, and the like) and/or other plants.

FIG. **3**A illustrates an exemplary suspension. In this example, an exemplary pond **300** has sides and a bottom, and is sufficiently deep to contain a suspension **310** at a depth **320**. Suspension **310** may be characterized by a surface that "faces" a source of light. In FIG. **3**A, a top surface of the suspension **310** faces light **330** (e.g., sunlight) arriving in an incident direction. The facing surface (e.g., the top surface) of suspension **310** may be characterized by an area **340**. Suspension **310** may include a liquid **350** and a suspended phase **360**.

Liquid **350** may include aqueous media such as water, seawater, fresh water, brackish water, growth media, and the like. Suspended phase **360** may include suspended photosynthetic organisms, such as algae, diatoms, and the like. Exemplary algae may include members of the genus *Nannochloropsis*. Exemplary diatoms may include members of the genera *Navicula, Amphora, Thallasiosira, Chaetoceros, Nitzschia, Cyclotella, Skeletonema, Phaeodactylum, Achnanthes, Coscinodiscus, Cylindrotheca, Pseudo-Nitzschia, Thalassionema, Hantzschia, Cymbella*, and/or *Psammodictyon*. Liquid **350** may include an aqueous liquid, such as water, seawater, synthetic seawater, brackish water, growth media, and the like.

FIG. **3**B is a schematic illustration of an exemplary variation in light intensity with depth through a plurality of cells (e.g., within a suspension). FIG. **3**B illustrates a relationship **370** between light intensity **120** and depth **320** under a set of conditions. For example, relationship **370** may represent a measured light intensity at various depths within suspension **310**, at a particular incident light intensity, for a given concentration of certain organisms in a liquid having a certain composition. A first intensity **380** may be associated with a shallow depth **382** (e.g., at or near the top surface), and may be a relatively brighter light condition. A second intensity **390** may be a lower intensity of light at a deeper depth **392**.

The light intensity within the suspension may scale with the incident light intensity. For example, first intensity **380** at mid-day on a sunny day in the tropics may correspond to an intensity in a saturation regime **140** (FIG. **1**) (e.g., intensity **162**, FIG. **1**), and second intensity **390** and may correspond to an intensity corresponding to a "light limited" regime of some organisms, such as linear regime **130** (FIG. **1**). On a less-bright day (e.g., a cloudy day), first intensity **380** may correspond to an intensity associated with threshold **150** (FIG. **1**) or even a linear regime **130**, and second intensity **390** may correspond to a light level below which photosynthesis may not occur. In some cases, second intensity **390** may correspond to a relatively dark "repair" intensity at which a photosynthetic cell may repair damage to itself. On a particular day and time, some cells may be "overexposed" to light, some cells may be "underexposed" to light, and some cells may receive an optimal amount of light (e.g., an intensity at or near threshold **150**).

Many photosynthetic organisms adapt or acclimate to different light conditions. In some cases, a cell residing for significant time (e.g., hours or days) at a weak intensity may adapt to those weak light conditions, and may increase its sensitivity to light. A cell residing at a high intensity (e.g., intensity **380** on a sunny day) may adapt to bright light conditions, and may decrease its sensitivity to light. In some cases, sensitivity may be adjusted by adjusting one or more light harvesting antennae (LHA). Sensitivity may be adjusted by adjusting a violaxanthin-chlorophyll-a protein (VCP).

A cell that has adapted to weak-light conditions may have a "sensitized" LHA. Exposing such a "sensitized" cell to bright light may saturate or "overpower" the LHA, which may result in a substantial portion of the incident bright light not being used for photosynthesis. In some cases, bright light may result in an increased amount of non-photochemical quenching (NPQ), and/or an increased ratio of NPQ to photosynthetic absorption. In some cases, cells that are adapted to weak intensity may be damaged by a high intensity.

A cell that has acclimated to intense light may not harvest as much weak light as a cell that has acclimated to weak light. In some cases, a cell may "reduce the gain" of a light harvesting antenna in response to intense light, which may allow for a relatively larger fraction of light to pass through the cell without being absorbed by the antenna. In some cases, a reduction in LHA sensitivity may be manifest as a reduced slope **132** (FIG. **1**) as compared to the slope characterizing a cell having a standard LHA (e.g., a wild type).

Cells nearer to an incident light source (e.g., at the top of a tree or top of a suspension) may absorb some incident light, and a portion of the incident light may pass through the cells to the "shaded" cells beneath or behind the nearer cells. Light that has passed through a first cell may be absorbed by a second cell. In some embodiments, an overall efficiency of a plurality of photosynthetic cells may be increased by reducing the amount of light absorbed each individual cell, and more particularly, by minimizing an amount of light that is absorbed via non-photosynthetic processes (e.g., NPQ). In some embodiments, a plurality of cells are engineered to have an increased transparency via a reduced LHA sensitivity. By minimizing the scattering and/or absorption of light via non-photosynthetic mechanisms (e.g., NPQ, dissipation of light as heat, ionization, damage, and the like), light that is not used for photosynthesis by a first cell may pass through the first cell be available for use by a second cell.

In some cases, a reduction in each individual cell's ability to harvest light may result in an increase in the overall efficiency of a population of the cells. A reduction in light harvesting efficiency results in greater transmittance of light through a cell, which increases the light available to other cells. A reduction in LHA efficiency may be manifest as an increased transparency of the cell (and/or a plurality of such cells).

FIG. **4** is a schematic illustration of an effect of increased transparency, according to some embodiments. FIG. **4** illustrates two variations in measured light intensity **120** as a function of depth **410** in two suspensions, and may be determined by measuring an intensity of light at various depths (or distances from the surface facing the light) of the suspension. A first organism WT **420** may be a native, wild type, or other unmodified organism. A second organism PGM **430** may include a modified transparency, and in some cases, may be characterized by an increased transparency as compared to WT **420**. A suspension of PGM **430** cells may attenuate light less than a corresponding suspension of WT **420** cells, as illustrated in FIG. **4**.

In some embodiments, transparency may be increased by reducing a size and/or number of LHA. In some cases, transparency may be increased by decreasing an amount of chlorophyll in the cell (e.g., an amount of chlorophyll associated with one or more LHA). In some cases, a transparency may be increased by decreasing an amount of chlorophyll in apparatus associated with Photosystem I. In some cases, a transparency may be increased by decreasing an amount of chlorophyll in apparatus associated with Photosystem II (PSII). In some cases a transparency may be increased by decreasing an amount of carotenoid (e.g., Violaxanthin) with and/or within a LHA.

FIG. **5** illustrates a schematic comparison of two photosynthetic rate responses, according to some embodiments. FIG. **5** is a schematic graph of photosynthetic rate **110** as a function of incident light intensity **120**. In some embodiments, Photosynthetic rate **110** is associated with a Photosystem II electron transport rate. FIG. **5** compares responses for two organisms, a wild type organism WT **420** and a modified organism PGM **430**. In some embodiments, PGM **430** may reach a higher photosynthetic rate **110** than WT **420**, at one or more light intensities. In some cases, PGM **430** has a higher maximum photosynthetic rate than does WT **420**. PGM **430** may have a linear regime characterized by a slope **532** that is lower than the corresponding slope **522** of WT **420**. In some cases, PGM **430** may have a threshold **550** that is higher than the corresponding threshold **560** of WT **420**. PGM **430** may have a higher slope at high light intensities (e.g., above threshold **540**) than does WT **420**. At some particularly high light intensities, the photosynthetic rate of WT **420** may decrease with increasing intensity, whereas the photosynthetic rate of PGM **430** at those intensities may not decrease as much or at all.

FIG. **6** illustrates a schematic comparison of light loss as a function of light intensity, according to some embodiments. Light loss **600** may characterize an amount of light incident on a cell that is not used for photosynthesis. Light loss **600** may be associated with various dissipation mechanisms, such as NPQ, a conversion of light to heat, photoinduced ionization, and the like. In some embodiments, an unmodified organism WT **420** may lose or otherwise dissipate more light than a modified organism PGM **430**, particularly at high light intensities.

FIG. **7** illustrates a schematic comparison of photochemical responses, according to some embodiments. Photochemical response **700** may include a response such as a Photosystem I electron transport rate. In some embodiments, WT **420** and PGM **430** may have similar photochemical responses **700**. In some cases, a WT **420** and a PGM **430** may display similar first photochemical responses (e.g., PS I electron transport rate) while having different second photochemical responses (e.g., PS II electron transport rate).

In some embodiments, PGM **430** may display a different photochemical response **700** as compared to WT **420** (e.g., higher slope and/or higher maximum). In some cases, this difference may increase with time (e.g., exposure time, growth time, replications, and the like). This difference may vary with irradiant intensity.

FIG. **8** illustrates a schematic comparison of transparency as a function of cell density, according to some embodiments. Transparency **800** may be measured as a function of incident light intensity, depth, cell density (e.g., number of cells per volume of liquid), and the like. Transparency may be measured using an apparatus having a defined path length and light intensity. Transparency may be measured by measuring light intensity at one or more points within a suspension as a function of incident intensity. Cell density measurements (e.g., cell counting over a volume) may be used to differentiate among factors affecting transparency (e.g., cell transparency vs. number of cells). In some cases, cell density **810** may include a response associated with cell replication (e.g., new cells growing) and/or adapting to light conditions (e.g., cell density). In some cases, cell density **810** may include a response associated with cells adapting to light (e.g., high cell densities and corresponding decreased mean intensities may result in adaptation of LHA apparatus).

At one or more cell densities **810**, modified organism PGM **430** may have an increased transparency as compared to unmodified organism WT **420**. Increased transparency may be manifest as an increased measured light intensity at a point within a suspension of modified organisms, as compared to an equivalent measurement (e.g., at equal cell density) within a suspension of unmodified organisms.

FIG. **9** illustrates a schematic comparison of transition thresholds of modified and unmodified cells, according to some embodiments. FIG. **9** schematically compares a response of a modified organism PGM **430** to that of an unmodified organism WT **420**. An exemplary transition is shown as threshold **150** in FIG. **9**, although other transitions (e.g., Ek) may be used. In some cases, a transition may characterize an irradiance level for optimal photosynthetic productivity. In some cases, increasing cell density may be associated with growth of cells.

FIG. **10** illustrates a schematic comparison of maximum photosynthetic rates, according to certain embodiments. FIG. **10** schematically compares a response of a modified organism PGM **430** to that of an unmodified organism WT **420**. In this example, extracted values of Pmax **152** are shown as a function of cell density **810**.

FIG. **11** illustrates an exemplary method. A starting (e.g., native or wild type) organism is chosen in step **1110**. In step **1120**, the organism is mutated (e.g., using mutagenesis) to create one or more mutated organisms. One or more properties of the organisms may be compared in step **1130**, which may include selecting one or more mutated organisms. Selecting may include using fluorescence activated cell sorting to select cells based on certain optical properties. Certain embodiments include screening based on phenotypes. In some cases, a specific genetic sequence may be modified (e.g., a genetic sequence that reduces an efficacy of a light harvesting antenna may be incorporated into an organism).

Properties to be screened for and compared may include transparency, threshold, Pmax, PSII properties, PSI properties, NPQ, and the like. In some cases, properties may include optical properties, and may include properties that may be rapidly screened for and/or measured. In some cases, organisms may be selected using quickly screenable properties (e.g., transparency). A selected subset may be further screened for properties that may take longer to evaluate (e.g., growth rates).

In optional step **1140**, growth rates may be compared. One or more mutated organisms may be selected in step **1150**. In some cases, a selected organism may have a higher growth rate than the wild type equivalent, and may also have certain properties (e.g., as determined in step **1130**) that distinguish it from the wild type organism. A selected mutated organism may have an increased transparency, a higher PSII ETR, a lower NPQ, a paler color, a different fluorescence spectrum and/or intensity, and the like.

In some embodiments, cells may be subjected to mutagenesis, and the mutated cells may be grown. Mutated cells having increased transparency as compared to native cells may be selected. In some cases, cells having increased transparency may be further selected based on growth rates (e.g., choosing those cultures with the highest growth rates). In certain cases, cultures having high growth rates under high light conditions and high cellular densities are selected.

EXAMPLE 1

Random Mutagenesis Using ICR-191

Nannochloropsis sp. (e.g., *Oceanica*) were mutated and their properties measured. ICR-191 was prepared as a stock solution at a concentration of 1 mg/ml in 0.1N filter sterilized HCl. Cells were grown to mid-log phase and diluted to 10^6 cells/ml. To 20 ml of the diluted culture 40 μl of the ICR-191 stock was added. Flasks were placed on a shaker and illuminated at 50 μmol quanta $m^{-2}s^{-1}$. Following 7 days of growth cells were washed twice with growth medium and then plated on agar plates. After 3-4 weeks of growth on plates relatively pale green colonies were selected, re-suspended in medium and then re-plated on fresh agar plates.

Fluorescence and Spectroscopic Analysis of Photosynthetic Function

Pulse amplitude modulated (PAM) fluorescence was recorded at the growth temperature of the culture using a Dual-PAM (Walz, Effeltrich, Germany). Samples were illuminated with visible light using the red LED built into the Dual-PAM. Samples were dark adapted in the sample chamber for a minimum of 10 min prior to all measurements. The actual photochemical efficiency of PSII at any given actinic irradiance was calculated as Fm'−Fs/Fm'. The relative PSII ETR was calculated as the product of the actual photochemical efficiency of PSII and the actinic irradiance. NPQ was measured as Fm−Fm'/Fm'. In addition to PSII ETR, simultaneous measurements of PSI ETR were made. The photochemical efficiency of PSI at any given actinic irradiance was calculated as 1−(Y(ND)+Y(NA)). PSI ETR was then calculated as the product of Y(I) and the actinic irradiance.

FIGS. **12**A, **12**B, and **12**C illustrate experimental results for wild type (annotated WT) and mutated (annotated **979**) samples after day 1 of growth. FIG. **12**A illustrates PSII electron transport rate (ETR) data as a function of irradiance. FIG. **12**B illustrates NPQ as a function of irradiance. FIG. **12**C illustrates PSI ETR as a function of irradiance.

FIGS. **13**A, **13**B, and **13**C illustrate experimental results for wild type (annotated WT) and mutated (annotated **979**) samples after day 2 of growth. FIG. **13**A illustrates PSII electron transport rate (ETR) data as a function of irradiance. FIG. **13**B illustrates NPQ as a function of irradiance. FIG. **13**C illustrates PSI ETR as a function of irradiance.

FIGS. **14**A, **14**B, and **14**C illustrate experimental results for wild type (annotated WT) and mutated (annotated **979**) samples after day 4 of growth. FIG. **14**A illustrates PSII electron transport rate (ETR) data as a function of irradiance. FIG. **14**B illustrates NPQ as a function of irradiance. FIG. **14**C illustrates PSI ETR as a function of irradiance.

FIGS. **15**A, **15**B, and **15**C illustrate experimental results for wild type (annotated WT) and mutated (annotated **979**) samples after day 7 of growth. FIG. **15**A illustrates PSII electron transport rate (ETR) data as a function of irradiance. FIG. **15**B illustrates NPQ as a function of irradiance. FIG. **15**C illustrates PSI ETR as a function of irradiance.

FIG. **16** illustrates a comparison of irradiance vs. cell density for wild type (annotated WT) and mutated (annotated **979**) samples. In this example, "irradiance" generally describes a measured light intensity at a point within the suspension for a given incident intensity. Samples were measured at different lengths of growth time, generally corresponding to the days shown in FIGS. **12-15**. Mutated sample **979** generally resulted in a more intense measured light intensity (higher irradiance) at a given cell density, as compared to the wild type sample WT.

FIG. **17** illustrates a comparison of measured Ek vs. cell density for wild type (annotated WT) and mutated (annotated **979**) samples. In this example, Ek was estimated using the PSII ETR results of FIGS. **12-15**. Mutated sample **979** generally displayed a higher Ek at a given cell density.

FIG. **18** illustrates a comparison of measured Pmax vs. cell density for wild type (annotated WT) and mutated (annotated **979**) samples. In this example, Pmax is the maximum observed photosynthetic rate, and was estimated using the PSII ETR results of FIGS. **12-15**. Mutated sample **979** generally displayed a higher Pmax at a given cell density.

Some embodiments include sensors to sense various parameters (e.g., light intensity, concentration, depth, photosynthetic rate, clarity, pH, mass, dielectric constant, transparency, opacity, time, date, and other characteristics). Apparatus may monitor various sensors, and systems may be actuated by automated controls (solenoid, pneumatic, piezoelectric, and the like). Some embodiments include a computer readable storage medium coupled to a processor and memory. Executable instructions stored on the computer readable storage medium may be executed by the processor to perform various methods described herein. Sensors and actuators may be coupled to the processor, providing input and receiving instructions associated with various methods. Certain instructions provide for closed-loop control of various parameters via coupled sensors providing input and coupled actuators receiving instructions to adjust parameters. Certain embodiments include materials. A biofuel may be synthesized from a carbohydrate, a lipid, and/or other biomass, which may be derived from cells and methods according to various embodiments.

The above description is illustrative and not restrictive. Many variations of the invention will become apparent to those of skill in the art upon review of this disclosure. The scope of the invention should, therefore, be determined not with reference to the above description, but instead should be determined with reference to the appended claims along with their full scope of equivalents.

What is claimed is:

1. A method for increasing cell density of photosynthetic organisms, the method comprising:

choosing a first photosynthetic organism having a first transparency and a first cell density associated with light transmission through the first photosynthetic organism;

subjecting the first photosynthetic organism to mutagenesis to create one or more second photosynthetic organisms;

determining a second transparency associated with light transmission through at least one of the second photosynthetic organisms; and

selecting one or more second photosynthetic organisms for which the second transparency is greater than the first transparency; the first photosynthetic organism being a member of genus Nannochloropsis and the selected one or more second photosynthetic organisms having a second cell density greater than the first cell density by at least 5%.

2. The method of claim **1**, further comprising:

determining a first growth rate of the first photosynthetic organism;

determining a second growth rate of at least one of the second photosynthetic organisms; and

selecting one or more second photosynthetic organisms having the second growth rate larger than the first growth rate.

3. The method of claim **2**, wherein at least one of the second photosynthetic organisms has the second growth rate larger than the first growth rate and has the second transparency greater than the first transparency.

4. The method of claim **2**, wherein one or more growth rates include a measurement of total biomass.

5. The method of claim **2**, wherein one or more growth rates include a measurement of lipid production.

6. The method of claim **1**, wherein the first transparency and the second transparency are determined in the visible light regime.

7. The method of claim **1**, wherein a color of the first photosynthetic organism is a first green, and at least one of the selected second photosynthetic organisms is a paler green than the first green.

8. The method of claim **1**, wherein at least one of the selected second photosynthetic organisms has a less effective light harvesting antenna than does the first photosynthetic organism.

9. The method of claim **1**, wherein at least one of the second photosynthetic organisms has a higher PSII electron transport rate than the first photosynthetic organism at a first irradiance.

10. The method of claim **9**, wherein the first irradiance is associated with a saturation regime of the first photosynthetic organism.

11. The method of claim **1**, wherein at least one of the second photosynthetic organisms has a lower value associated with nonphotochemical quenching than does the first photosynthetic organism at a first irradiance.

12. The method of claim **1**, wherein at least one of the second photosynthetic organisms has a higher threshold irradiance than does the first photosynthetic organism.

13. The method of claim **1**, wherein at least one of the second photosynthetic organisms has a higher maximum photosynthetic rate than does the first photosynthetic organism.

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